Factors Limiting the Efficacy of Hydrogen Peroxide Washes for Decontamination of Apples Containing *Escherichia coli*

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ABSTRACT: Factors limiting efficacy of H_2O_2 washes and alternative decontamination strategies were investigated with Golden Delicious apples, inoculated with nonpathogenic *Escherichia coli*. Post-treatment rinsing decreased efficacy by eliminating residual H_2O_2. A 2-stage wash incorporating a rinse to remove surfactant residues prior to H_2O_2 application was developed. Rapid attachment of *E. coli* to apples prevented effective removal by washing with water. Surviving *E. coli* following a 5% H_2O_2 wash were concentrated in stem and calyx areas. Survival was independent of the time interval between inoculation and washing. *E. coli* inoculation of punctured apple surfaces resulted in growth at 20 °C and greater survival after washing with 5% H_2O_2. Improved decontamination methods are needed.

Key Words: *Escherichia coli*, apples, decontamination, washing, sanitizers, hydrogen peroxide

Introduction

RECENT OUTBREAKS OF FOODBORNE ILLNESS associated with *Escherichia coli* O157:H7 in unpasteurized apple cider (Besser and others 1993; CDC 1996; CDC 1997) have focused attention on the need for effective treatments to decontaminate fresh apples that might contain this or other human pathogens. Recent studies in our laboratory (Sapers and others 1999) have demonstrated the inability of washes containing chlorine or commercial sanitizing agents for fruits and vegetables to reduce populations of generic *E. coli* on inoculated apple surfaces by more than 1 to 2 $\log_{10}$CFU/g when apples were immersed in the wash solution for 1 min with agitation. In contrast, washing with solutions containing hydrogen peroxide (H_2O_2), alone or with commercial sanitizing agents, achieved population reductions as great as 3 to 4 $\log_{10}$CFU/g. However, the 5-log reduction proposed by the U.S. Food and Drug Administration for fruit and vegetable juices and juice products (FDA 1998) could not be attained by washing. Also, under some laboratory and field conditions, even the most promising experimental washes were only marginally effective (Sapers and Buchanan 1997; Sapers and Jantschke 1998).

These shortcomings indicate the need for further research to elucidate factors limiting the efficacy of washing treatments. Therefore, studies were carried out to investigate constraints on decontaminating apples by washing and to suggest alternative strategies when required.

Results and Discussion

Effect of rinsing after application of an antimicrobial wash

In preliminary H_2O_2 washing trials with apple halves inoculated with *E. coli* O157:H7 (Sapers and Buchanan 1997), we found that treatments expected to produce a population reduction of 3 to 4 $\log_{10}$CFU/g, based on earlier work with nonpathogenic *E. coli* (Sapers and others 1999), did not exceed 1.5 $\log_{10}$CFU/g. The principal difference between the 2 studies was the inclusion of a rinsing step after application of the antimicrobial wash in the *E. coli* O157:H7 trials, which was not done in the earlier trials. Similar results were obtained in washing trials carried out at the National Food Processors Association (NFPA) facility with whole apples rinsed after application of the wash solution (data not shown). A side-by-side comparison of rinsed and nonrinsed samples confirmed that rinsing the inoculated apples with water after washing with 5% H_2O_2 reduced the ability of the treatments to lower the bacterial population (Table 1). With the combination of 5% H_2O_2 and APL-Kleen® 246, the rinsing effect was marginally significant. We suspected that when washed apples were homogenized, residual H_2O_2 from the wet apple surfaces killed bacteria that were released from inaccessible sites near the stem and calyx where they had been protected from exposure to peroxide in the bulk wash solution. Rinsing reduced the concentration of residual H_2O_2 on apple halves from about 1000 mg/L to only 20 to 50 mg/L, not enough to exert much additional lethal effect.

Commercial surfactant formulations might be used in combination with H_2O_2 treatments to remove soil and pesticide residues from fruit surfaces, but surfactant or other chemical residues would have to be removed by rinsing. This would be incompatible with the need to retain a small peroxide residue. Therefore, we developed an alternative means of washing ap-

Table 1—Effect of rinsing on efficacy of H_2O_2-based wash for decontamination of Golden Delicious apple halves inoculated with *E. coli* (ATCC 25922)

Treatment	Rinse	$\log_{10}$CFU/g[a]	T-test[b]	H_2O_2 residue (mg/L)[c]
Inoculated control	—	4.91 ± 0.25	—	—
5% H_2O_2	No	2.16 ± 0.20	0.05	690
	Yes	3.07 ± 0.24		18
5% H_2O_2 + 1% APL-Kleen®245				
	No	1.97 ± 0.17	0.10	1040
	Yes	2.72 ± 0.58		54
5% H_2O_2 +1% APL-Kleen® 245				
	No	2.46 ± 0.27	NS	40
	Yes	2.79 ± 0.31		30

[a]Mean of 3 independent trials.
[b]Within each treatment, significance of difference in $\log_{10}$CFU/g between rinse and no rinse determined by t-test at significance level shown; NS=not significant.
[c]Determined within 2 min of treatment application during one of trials.

ples with surfactants and H_2O_2 in 2 stages that avoided removal of residual peroxide. In the 1st stage, a commercial acidic surfactant formulation (APL-Kleen® 245 or 246) or trisodium phosphate (TSP) was applied at 50 °C to remove soil and then was rinsed off with water. In the 2nd stage, 5% H_2O_2 was applied at 50 °C without rinsing so that residual peroxide would be available to kill surviving *E. coli* when the fruit was homogenized before breaking down to water and oxygen. Results of washing trials indicated that the 2-stage treatment with APL-Kleen® 245 was at least as effective as a single stage wash containing both the acidic surfactant and H_2O_2 or H_2O_2 alone in reducing the population of *E. coli* on inoculated apples (Table 2). Results were more erratic when the H_2O_2 wash was preceded by a TSP wash and water rinse (standard deviation of $\log_{10}$ CFU/g reduction = 1.12), and significant differences could not be demonstrated. This may have resulted from the presence of a TSP residue after rinsing that accelerated the breakdown of the peroxide, as reported (Sapers and others 1999). The combination of TSP and H_2O_2 was not tested in this experiment because of the aforementioned incompatibility. Improvements in treatment efficacy resulting from use of a 2-stage treatment may be applicable to commercial production of apple cider where the washed apples would be disintegrated in a hammer mill prior to pressing, a sequence analogous to sample homogenization prior to microbiological evaluation in the laboratory protocol.

Table 2—Efficacy of 2-stage washes with commercial sanitizers and H_2O_2 in decontaminating whole Golden Delicious apples[a]

Expt.	Treatment[b]	n	$\log_{10}$CFU/g reduction[c]
A	5% H_2O_2	4	2.34[ef]
	1% APL-Kleen® 245	4	1.81[f]
	1% APL-Kleen® 245 + 5% H_2O_2	4	2.41[e]
	1% APL-Kleen® 245; rinse; 5% H_2O_2[d]	4	2.73[e]
B	5% H_2O_2	6	2.22[e]
	4% trisodium phosphate	6	2.08[e]
	4% trisodium phosphate; rinse; 5% H_2O_2[d]	6	2.85[e]

[a]Inoculated with *E. coli* (ATCC 25922).
[b]1 min wash at 50 °C.
[c]Based on $\log_{10}$CFU/g of corresponding inoculated controls (mean = 5.97 ± 0.18 for Expt. A and 4.52 ± 0.12 for Expt. B).
[d]Two-stage treatment: acidic surfactant or trisodium phosphate wash followed by 5% H_2O_2 wash with intermediate rinse.
[e-f]Within experiments, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

Table 3—Attachment of *E. coli* (ATCC 25922) to apple surfaces

Time after inoculation (h)	$\log_{10}$CFU/g[a] 4 °C Inoculated control	4 °C After wash	20 °C Inoculated control	20 °C After wash[e]	$\log_{10}$CFU/g reduction from wash 4 °C	20 °C
0.5	4.40[b]	3.46[b]	4.35[bc]	3.38[d]	0.94*	0.97**
24	3.89[bc]	3.22[b]	4.80[b]	4.33[bc]	NS	0.47**
48	3.88[bc]	3.97[b]	4.06[c]	4.65[b]	NS	-0.59**
72	3.66[c]	3.64[b]	4.18[bc]	3.88[cd]	NS	NS

[a]Mean of duplicate trials.
[b-d]Within the same column, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.
[e]Significance of $\log_{10}$CFU/g reduction tested by ANOVA: * ($p < 0.05$), ** ($p < 0.01$), NS=not significant.

Table 4—Effect of interval between inoculation and washing on efficacy of sanitizing washes in decontaminating whole Golden Delicious apples inoculated with *E. coli* (ATCC 25922)

Storage Temp. (°C)	Storage Time (h)	$\log_{10}$CFU/g Control	200 mg/L Cl_2[a]	5% H_2O_2 at 50 °C[a]
20	0.5	6.30[b]	4.20[b]	4.17[b]
	24	5.00[b]	4.40[b]	3.70[b]
	48	4.87[b]	4.55[b]	3.98[b]
	72	3.08[b]	3.52[b]	2.88[b]
4	0.5	6.16[b]	4.31[bc]	3.68[c]
	24	5.85[b]	4.54[b]	3.80[b]
	48	5.67[b]	4.66[c]	3.98[c]
	72	5.66[b]	4.52[b]	4.16[b]

[a]2-min wash.
[b-c]Within the same row, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

Control of foam

Washing trials with 2% APL-Kleen® 246, carried out with the Vanmark peeler/washer at the NFPA facility, demonstrated that foam production could be a limiting factor. In these trials, foam could not be controlled, even by addition of 1760 mg/L Dow Corning FG-10 antifoam, a level greatly exceeding that permitted by FDA (21 CFR 173.340). Attempts in our laboratory to control foaming of 1% to 2% APL-Kleen® 246 by addition of other antifoam agents were not successful. However, an alternative sanitizing agent, 1% APL-Kleen® 245, showed a much lower tendency to foam than APL-Kleen® 246. Shaking trials carried out with this sanitizer demonstrated that as little as 100 mg/L of FG-10 was effective in completely suppressing foam production. Addition of 100 mg/L FG-10 to 5% H_2O_2 (50 °C) had no adverse effect on the efficacy of this wash in reducing the population of *E. coli* on inoculated apple halves (data not shown). In a 2nd series of trials carried out at the NFPA facility, foam production by 1% APL-Kleen® 245 in the Vanmark peeler/washer was minimal, even when no antifoam agent was added.

Bacterial attachment to apple surfaces

One of the factors limiting the efficacy of washing as a means of decontaminating apples is the attachment of bacterial cells to product surfaces. We studied the rapidity and extent of *E. coli* attachment to Golden Delicious apples that were inoculated by immersion in a bacterial suspension, drained, and then held in air at 4 or 20 °C for various times. In these trials, we measured the survival of *E. coli* on the inoculated apples (controls) and also the numbers of *E. coli* remaining after the apples had been washed with water at ambient temperature using the standardized washing procedure described above. During storage for 72 h, there was little or no change in the bacterial population on inoculated control apples at 4 and 20 °C (Table 3). When apples were held for 30 min after inoculation and then washed, about 90% (1 $\log_{10}$ CFU/g) of the bacteria could be removed. When apples were held for 24 h after inoculation, the population reduction was substantially less (marginally significant). For holding times in excess of 24 h, washing was completely ineffective in removing bacteria. Whether this is a reflection of physical adhesion of bacterial cells to the apple surface or incorporation in a biofilm is not clear. However, the end result is a sharp reduction in the efficacy of washing. Thus, superficial "washing" by simple immersion of apples in a wet dump tank or by low pressure spraying with water, as is sometimes practiced, is unlikely to remove well-established bacterial contaminants. However, such washing might still be effective and useful in removing dirt, pesticide residues, and loosely attached microbial contaminants.

The time interval between inoculation and washing had no effect on the size of the surviving bacterial population following washes with 200 mg/L Cl_2, applied at room temperature, or with 5% H_2O_2 at 50 °C (Table 4). However, because of some fluctuation in the inoculated control population during storage at both 4 and 20 °C, apparent values of $\log_{10}$ reduction (CFU/g) due to washing were erratic or even negative. In such cases, the interpretation of treatment efficacy data might be confusing. These results suggest that a substantial part of the *E. coli* population is attached to apple surfaces in such a way as to permit survival during washing with antimicrobial agents.

A further complication of bacterial attachment is the nonuniform distribution of *E. coli* on the surface of inoculated apples. In a study of apples that had been segmented and cored 24

Table 5—Distribution of *E. coli* (ATCC 25922) on surface of inoculated apples before and after washing with 5% H_2O_2 at 50 °C

Location	Log_{10}(CFU/cm²)[a] 24h after inoculation Inoculated	Washed[b]	72h after inoculation Inoculated	Washed[b]
Skin on wedges	4.77[d]	2.05[d]	4.37[d]	1.63[d]
Skin at calyx end of core	7.26[c]	5.20[c]	6.79[c]	4.46[c]
Skin on stem end of core	6.63[c]	5.06[c]	5.61[c]	4.89[c]

[a]Based on calculated surface area of skin.
[b]Washed 1 min in 5% H_2O_2 at 50 °C.
[c-d]Within the same column, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

Table 6—Growth of *E. coli* and *E. aerogenes* in punctures on inoculated Golden Delicious apples[a]

Exp.	Strain	No. of punctures	Inoculum strength (log_{10}CFU/mL)	Log_{10}CFU/g[a] Time after inoculation (h) 0.5	24	48
C	*E. coli* ATCC 25922	4[b]	7.24	4.85[f]	6.03[e]	ND[d]
	E. coli ATCC 23716	4	7.11	4.78[f]	5.54[e]	ND
	E. coli ATCC 11775	4	7.40	5.37[f]	6.10[e]	ND
	E. aerogenes B199	4	7.10	5.28[f]	6.14[e]	ND
D	*E. coli* ATCC 25922	1[c]	6.40	3.53[f]	4.85[e]	4.96[e]

[a]Based on weight of whole apple; mean of duplicate trials.
[b]Four 1-cm deep punctures made with a 6.5-mm dia sterile nail on opposite sides of apple on equator.
[c]1-cm deep puncture made with 3.7-mm dia sterile nail on top of apple 2 to cm from stem.
[d]ND=not determined.
[e-f]Within the same row, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

Table 7—Efficacy of H_2O_2-based washes for decontamination of punctured Golden Delicious apples inoculated with *E. coli* (ATCC 25922)[a]

Treatment	Log_{10}CFU/g reduction[b]
5% H_2O_2	0.58[f]
1% APL-Kleen® 245; 5% H_2O_2	1.62[e]
1% APL-Kleen® 245; 5% H_2O_2; 1000 mg/L H_2O_2[c]	2.60[d]

[a]1-cm deep puncture made with 3.7-mm dia sterile nail on top of apple 2 to 3 cm from stem.
[b]Based on control populations of 4.88 log_{10}CFU/g. Means of duplicate trials.
[c]Two-stage treatment followed by addition of 1000 mg/L H_2O_2 to homogenate.
[d-f]Within the same column, means with no letter in common are significantly different ($p < 0.05$) by Bonferroni LSD.

h after inoculation, we found that the bacterial population per cm² of skin surface was more than 2 logs greater on skin attached to the stem and calyx areas than on the skin surface of the wedges (Table 5). When the inoculated apples were washed with 5% H_2O_2 at 50 °C, the bacterial population was reduced to ≤ 2 log_{10}CFU/cm² on skin of wedges in contrast to approximately 5 log_{10}CFU/cm² on skin in the calyx and core areas. Similar results were obtained after 72 h. The ability of *E coli* to bind preferentially to the apple surface near the stem and calyx ends greatly complicates the problem of washing because commonly used fruit washing systems are not designed to scrub or direct jets of water or detergent solution in these areas of the apple. Such a modification of washing equipment might improve the efficacy of washing in reducing bacterial populations on apples.

Effects of contaminated punctures

It is not unusual to find apples with skin punctures in raw material for processing or even in some fruit intended for fresh market. Such damage can occur from hail or bird pecks prior to harvest or can result from contact with stems of adjacent apples or splinters in wooden bins during harvesting and handling. The presence or even growth of *E. coli* within skin punctures, as might result from exposure to contaminated water during irrigation or in a drencher, dump tank, or flume could greatly complicate the problem of decontamination by washing. We found that bacterial growth did occur in punctured apples (4 punctures/apple) that were inoculated with *E. coli* (3 strains) or *E. aerogenes* B199, with the population increasing by 0.7 to 1.2 log_{10}CFU/g in 24 h at 20 °C. Even in apples having only 1 puncture, the population increased from an initial level of 4.4 log_{10}CFU/g to 5.2 log_{10}CFU/g over 48 h (Table 6). These populations are based on the whole apple weight and would be substantially higher in the area of the puncture. Growth did not occur when the inoculated apples were incubated at 4 °C (data not shown). Janisiewicz and others (1999) recently reported exponential growth of *E. coli* O157:H7 and 2 nonpathogenic *E. coli* strains in artificial wounds (3-mm dia punctures) in Golden Delicious apple. Populations reached 5 to 7 log_{10}CFU/wound within 48 h at ambient temperature, depending on the strain and inoculum concentration.

Washing trials with inoculated punctured apples demonstrated the limited ability of antimicrobial washes to kill or remove *E. coli* within punctures (Table 7). Population reductions obtained with 1- or 2-stage wash treatments with 5% H_2O_2 at 50 °C were ≤ 1.6 log_{10}CFU/g, in contrast to population reductions of 2 to 3 log_{10}CFU/g usually obtained with inoculated intact apples (see Table 2 and Sapers and others 1999). The population reduction could be increased by addition of 1000 mg/L H_2O_2 to the washed apples during homogenization. This result is analogous to the residual peroxide effect observed in washed, inoculated apple halves, with and without a post-treatment rinse. However, it probably would not be feasible to add H_2O_2 to disintegrated apples or juice during commercial cider production because of the complexity of the process, possible loss of quality, and regulatory constraints.

The problem of contaminated punctures is best addressed by minimizing exposure of apples to sources of microbial contamination through use of good agricultural practices and through careful sorting of raw material to exclude apples with punctures. Surface pasteurization with hot water or steam might kill bacteria within shallow punctures without imparting an undesirable cooked flavor to the juice (Fallik and others 1998), but the efficacy of this treatment has not yet been established, and surface pasteurization would be compromised by the presence of human pathogens within the core tissue (Buchanan and other 1999). If surface pasteurization is not sufficient, the juice should be heat pasteurized, a widely used option, or pasteurized by irradiation with ultraviolet light (McCandless 1998), a promising alternative yet to be approved.

Other technologies that have the potential of achieving pasteurization of juice are use of high pressure treatments and pulsed electric fields. However, these and other nonthermal pasteurization treatments are probably too expensive for use with apple cider.

Conclusions

NONPATHOGENIC STRAINS OF *E. COLI* WERE EMPLOYED IN THESE experiments rather than *E. coli* O157:H7 to allow us to test treatments and employ sample handling conditions that would be difficult to carry out safely if the human pathogen were used. Our results demonstrated that the efficacy of washing as a means of decontaminating artificially inoculated apples is limited by microbial adhesion to apple surfaces, attachment at inaccessible sites, and survival and growth in punctures. Further studies to overcome these limiting conditions also are being conducted with surrogates since the treatments are difficult to contain. However, confirmatory trials with *E. coli* O157:H7, applied under conditions simulating natural contamination, will be required to validate promising new treatments.

Materials and Methods

Preparation and inoculation of apples

Unwaxed Golden Delicious apples of known origin were obtained from produce distributors and stored at 4 °C for no more than 3 mo until needed. Apples for inoculation were either uncut; cut in half along the core axis; or punctured with a sterile nail to produce a 1-cm deep hole (either 1 puncture made with a 3.7-mm dia nail 2 to 3 cm from stem, or 4 punctures made with a 6.5-mm dia nail at the stem end, calyx end, and at opposite sides on the apple equator). Sets of 9 whole or punctured apples or 18 halves were immersed for 5 min in an inoculum containing approximately 1.3×10^7 CFU/mL of a nonpathogenic *E. coli* (ATCC 11775, 23716, or 25922) or *Enterobacter aerogenes* (B199). Apples were drained and air-dried at 4 °C or ambient temperature (about 20 °C) for 30 min, 24 h, 48 h, or 72 h. In some trials, the inoculum concentration was increased to about 3.5×10^8 CFU/mL so that a 5-log reduction in the *E. coli* population on inoculated apples could be demonstrated.

Washing trials

In washing trials, inoculated whole apples, punctured apples, or apple halves were decontaminated by washing in 4 L of sanitizer solution at 50 °C on a shaker for 1 min, as described (Sapers and others 1999). Sanitizing solutions included 200 mg/L Cl_2 (pH adjusted to 6.5 with citric acid, applied at about 20 °C instead of 50 °C), 1% or 2% APL-Kleen® 245 or 246 (Elf Atochem North America Inc., Agrichemicals Div., Decco Dept., Monrovia, Calif., U.S.A.), 4% trisodium phosphate (TSP) (Rhodia, Inc.), 5% H_2O_2, and combinations of 5% H_2O_2 with APL-Kleen® 245 or 246. In some trials, the decontamination treatment was applied in 2 stages: a wash with APL Kleen® 245 or TSP, as described above (1st stage), followed by draining, rinsing with tap water in a colander for 10 s, and a 2nd wash with a H_2O_2 solution (2nd stage). Following treatment, the apples were drained, in some trials rinsed with water as described above, subdivided, and homogenized with 2 L sterile 0.1% peptone (Difco) for 1 min at medium speed in a 1-gal stainless steel blending container (Waring Heavy Duty Lab Blender Model 38BL52; Waring Products Div., Dynamics Corp. of America, New Hartford, Conn., U.S.A.). In some trials with inoculated whole apples, each of 6 replicate, similarly sized apples was subdivided into 10 wedges and a 2-cm dia core with a stainless steel kitchen apple slicer (Westmark Divisorex, Model 5110) after storage at 4 °C for 24 or 72 h following inoculation. Stem and calyx ends of the core were removed with a sterile knife. All like fractions were pooled, weighed, blended, and sampled for enumeration of *E. coli*. The total skin surface area of wedges, calyx, and stem portions were each estimated from the apple dimensions so that counts could be expressed as CFU/cm² surface area. This procedure was repeated on apples that were held for 24 or 72 h after inoculation and then washed with 5% H_2O_2 at 50 °C. In some trials with punctured apples, additional H_2O_2 was added to apple homogenates after application of a 2-stage wash so that the residual concentration would be about 1000 mg/L. Homogenates were diluted with 0.1% peptone for plating on Petrifilm *E. coli* Count Plates (3M Microbiology Products, St. Paul, Minn., U.S.A.) and Brain Heart Infusion Agar. Plates were incubated for 24 h at 37 °C.

The effectiveness of antifoam agents in suppressing foam generated by combinations of 5% H_2O_2 and 1% to 2% commercial sanitizers was determined by addition of 10 mg/L SBI Antifoam (Systems Bio-Industries Inc., Waukesha, Wis., U.S.A.) or Dow Corning 1510-US, 1520-US or FG10 antifoam emulsions (Dow Corning Corp., Midland, Mich., U.S.A.). Foam was produced by blending 250 mL of these solutions at low speed with a Waring Blender for 5 s or shaking 50 mL in 225 mL bottles on a Wrist Action Shaker (Model 175, Burrell Corp., Pittsburgh, Pa., U.S.A.) for 10 to 30 s at an amplitude control setting of 10, and the height of foam was recorded. Apple washing trials were carried out with and without antifoam addition to determine whether such agents affected treatment efficacy.

Field test of washing procedures

Preliminary field tests of washing procedures developed in the laboratory were conducted at National Food Processors Assn. Center for Technical Assistance (Dublin, Calif., U.S.A.). Laboratory procedures were scaled up to permit washing of 45.4 kg quantities of orchard run Golden Delicious or Granny Smith apples that had been inoculated with *E. coli* (ATCC 25922). Washing treatments comparable to those described above were applied in a Vanmark Model 2500 Peeler/Washer (Vanmark Corp., Creston, Iowa, U.S.A.). Subsamples (1500 g) of treated apples and controls were homogenized, diluted with 0.1% peptone water, and plated on Plate Count agar and EMB agar (Difco Laboratories, Detroit, Mich., U.S.A.).

Hydrogen peroxide residues in washed apples

Homogenates of treated apples were analyzed for peroxide by the Reflectoquant analysis system (EM Science, Gibbstown, N.J., U.S.A.) over several hours. Similar measurements were made on juice prepared from homogenates of treated apples by straining through cheesecloth.

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